APPLICATION

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TITLE: **FATTY ACID ELONGASES**

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FATTY ACID ELONGASES

Field of the Invention

This invention relates to fatty acid elongase complexes and nucleic acids encoding elongase proteins. More particularly, the invention relates to nucleic acids encoding ß-keto acyl synthase proteins that are effective for producing very long chain fatty acids, polypeptides produced from such nucleic acids and transgenic plants expressing such nucleic acids.

Background of the Invention

Plants are known to synthesize very long chain fatty acids (VLCFAs). VLCFAs are saturated or unsaturated monocarboxylic acids with an unbranched even-numbered carbon chain that is greater than 18 carbons in length. Many VLCFAs are 20-32 carbons in length, but VLCFAs can be up to 60 carbons in length. Important VLCFAs include erucic acid (22:1, i.e., a 22 carbon chain with one double bond), nervonic acid (24:1), behenic acid (22:0), and arachidic acid (20:0).

Plant seeds accumulate mostly 16- and 18-carbon fatty acids. VLCFAs are not desirable in edible oils. Oilseeds of the *Crucifereae* (e.g., rapeseed) and a few other plants, however, accumulate C20 and C22 fatty acids (FAs). Although plant breeders have developed rapeseed lines that have low levels of VLCFAs for edible oil purposes, even lower levels would be desirable. On the other hand, vegetable oils having elevated levels of VLCFAs are desirable for certain industrial uses, including uses as lubricants, fuels and as a feedstock for plastics, pharmaceuticals and cosmetics.

The biosynthesis of saturated fatty acids up to an 18-carbon chain occurs in the chloroplast. C2 units from acyl thioesters are linked sequentially, beginning with the condensation of acetyl Coenzyme A (CoA) and malonyl acyl carrier protein (ACP) to form a C4 acyl fatty acid. This condensation reaction is catalyzed by a ß-ketoacyl synthase III (KASIII). ß-ketoacyl moieties are also referred to as 3-ketoacyl moieties.

The enzyme ß-ketoacyl synthase I (KASI) is involved in the addition of C2 groups to form the C6 to C16 saturated fatty acids. KASI catalyzes the stepwise condensation of a fatty acyl moiety (C4 to C14) with malonyl-ACP to produce a 3-ketoacyl-ACP product that is 2 carbons longer than the substrate. The last condensation reaction in the chloroplast, converting C16 to C18, is catalyzed by ß-ketoacyl synthase II (KASII).

Each elongation cycle involves three additional enzymatic steps in addition to the condensation reaction as discussed above. Briefly, the ß-ketoacyl condensation product is reduced to ß-hydroxyacyl-ACP, dehydrated to the enoyl-ACP, and finally reduced to a fully reduced acyl-ACP. The fully reduced fatty acyl-ACP reaction product then serves as the substrate for the next cycle of elongation.

The C18 saturated fatty acid (stearic acid, 18:0) can be transported out of the chloroplast and converted to the monounsaturate C18:1 (oleic acid), and the polyunsaturates C18:2 (linoleic acid) and C18:3 (α-linolenic acid). C18:0 and C18:1 can also be elongated outside the chloroplast to form VLCFAs. The formation of VLCFAs involves the sequential condensation of two carbon groups from malonyl CoA with a C18:0 or C18:1 fatty acid substrate. Elongation of fatty acids longer than 18 carbons depends on the activity of a fatty acid elongase complex to carry out four separate enzyme reactions similar to those described

above for fatty acid synthesis in the chloroplast. Fehling, Biochem. Biophys. Acta 1082:239-246 (1991). In plants, elongase complexes are distinct from fatty acid synthases since elongases are extraplastidial and membrane bound.

Mutations have been identified in an *Arabidopsis* gene associated with fatty acid elongation. This gene, designated the *FAE1* gene, is involved in the condensation step of an elongation cycle. See, WO 96/13582, incorporated herein by reference. Plants carrying a mutation in *FAE1* have significant decreases in the levels of VLCFAs in seeds. Genes associated with wax biosynthesis in jojoba have also been cloned and sequenced (WO 95/15387, incorporated herein by reference).

Very long chain fatty acids are key components of many biologically important compounds in animals, plants, and microorganisms. For example, in animals, the VLCFA arachidonic acid is a precursor to many prostaglandins. In plants VLCFAs are major constituents of triacylglycerols in many seed oils, are essential precursors for cuticular wax production, and are utilized in the synthesis of glycosylceramides, an important component of the plasma membrane.

Obtaining detailed information on the biochemistry of KAS enzymes has been hampered by the difficulties encountered when purifying membrane bound enzymes. Although elongase activities have been partially purified from a number of sources, or studied using cell fractions, the elucidation of the biochemistry of elongase complexes has been hampered by the complexity of the membrane fractions used as the enzyme source. For example, until recently, it was unclear as to whether plant elongase complexes were composed of a multifunctional polypeptide similar to the FAS found in animals and yeast, or if the complexes existed as

discrete and dissociable enzymes similar to the FAS of plants and bacteria. Partial purification of an elongase KAS, immunoblot identification of the hydroxy acyl dehydrase, and the recent cloning of a KAS gene (*FAE1*) suggest that the enzyme activities of elongase complexes exist on individual enzymes.

Summary of the Invention

The invention disclosed herein relates to an isolated polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above sequences that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

Also disclosed herein is an isolated polypeptide that has an amino acid sequence substantially identical to one of the following: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. Also disclosed are isolated polynucleotides encoding polypeptides substantially identical in their amino acid sequence to: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14.

The invention also relates to a transgenic plant containing a nucleic acid construct. The nucleic acid construct comprises a polynucleotide described above. The construct further comprises a regulatory element operably linked to the polynucleotide. The regulatory element may a

tissue-specific promoter, for example, an epidermal cell-specific promoter or a seed-specific promoter. The regulatory element may be operably linked to the polynucleotide in sense or antisense orientation. The plant has altered levels of very long chain fatty acids in tissues where the polynucleotide is expressed, compared to a parental plant lacking the nucleic acid construct.

A method is disclosed for altering the levels of very long chain fatty acids in a plant. The method comprises the steps of creating a nucleic acid construct and introducing the construct into the plant. The construct includes a polynucleotide selected from one of the following: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:13; an RNA analog of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, or 15; and a polynucleotide having a nucleic acid sequence complementary to one of the above. The polynucleotide can also be a nucleic acid fragment of one of the above that is at least 15 nucleotides in length and that hybridizes under stringent conditions to genomic DNA encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14. The polynucleotide is effective for altering the levels of very long chain fatty acids in the plant.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Figure 1 shows the time course of *in vitro* VLCFA synthesis by *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

Figure 2 shows the rates of *in vitro* VLCFA synthesis and the VLCFA profiles of *FAE1* expressed in yeast, with 3 different acyl-CoA substrates.

Figure 3 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL1 polynucleotide (SEQ ID NO:1).

Figure 4 shows the deduced amino acid sequence (SEQ ID NO:2) for the EL1 coding sequence of Figure 3.

Figure 5 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL2 polynucleotide (SEQ ID NO:3).

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:4) for the EL2 coding sequence of Figure 5.

Figure 7 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL3 polynucleotide (SEQ ID NO:5).

Figure 8 shows the deduced amino acid sequence (SEQ ID NO:6) for the EL3 coding sequence of Figure 7.

Figure 9 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL4 polynucleotide (SEQ ID NO:7).

Figure 10 shows the deduced amino acid sequence (SEQ ID NO:8) for the EL4 coding sequence of Figure 9.

Figure 11 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL5 polynucleotide (SEQ ID NO:9).

Figure 12 shows the deduced amino acid sequence (SEQ ID NO:10) for the EL5 coding sequence of Figure 11.

Figure 13 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL6 polynucleotide (SEQ ID NO:11).

Figure 14 shows the deduced amino acid sequence (SEQ ID NO:12) for the EL6 coding sequence of Figure 13.

Figure 15 shows the nucleotide sequence of the coding region of the *Arabidopsis* EL7 polynucleotide (SEQ ID NO:13).

Figure 16 shows the deduced amino acid sequence (SEQ ID NO:14) for the EL7 coding sequence of Figure 15.

Description of the Preferred Embodiments

The present invention comprises isolated nucleic acids (polynucleotides) that encode polypeptides having ß-ketoacyl synthase activity. The novel polynucleotides and polypeptides of the invention are involved in the synthesis of very long chain fatty acids and are useful for modulating the total amounts of such fatty acids and the specific VLCFA profile in plants.

A polynucleotide of the invention may be in the form of RNA or in the form of DNA, including cDNA, synthetic DNA or genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded, can be either the coding strand or non-coding strand. An RNA analog may be, for example, mRNA or a combination of ribo- and deoxyribonucleotides. Illustrative examples of a polynucleotide of the invention are shown in Figs. 3, 5, 7, 9, 11, 13 and 15.

A polynucleotide of the invention typically is at least 15 nucleotides (or base pairs, bp) in length. In some embodiments, a polynucleotide is about 20 to 100 nucleotides in length, or about 100 to 500 nucleotides in length. In other embodiments, a polynucleotide is greater than about 1500 nucleotides in length and encodes a polypeptide having the amino acid sequence shown in Figs. 4, 6, 8, 10, 12, 14 or 16.

In some embodiments, a polynucleotide of the invention encodes analogs or derivatives of a polypeptide having the deduced amino acid sequence of Figs. 4, 6, 8, 10, 12, 14 or 16. Such fragments, analogs on derivatives

include, for example, naturally occurring allelic variants, non-naturally occurring allelic variants, deletion variants and insertion variants, that do not substantially alter the function of the polypeptide.

A polynucleotide of the invention may further comprise additional nucleic acids. For example, a nucleic acid fragment encoding a secretory or leading amino acid sequence can be fused in-frame to the amino terminal end of one of the EL1 through EL7 polypeptides. Other nucleic acid fragments are known in the art that encode amino acid sequences useful for fusing in-frame to the KAS polypeptides disclosed herein. See, e.g., U.S. 5,629,193 incorporated herein by reference. A polynucleotide may further comprise one or more regulatory elements operably linked to a KAS polynucleotide disclosed herein.

The present invention also comprises polynucleotides that hybridize to a KAS polynucleotide disclosed herein. Such a polynucleotide typically is at least 15 nucleotides in length. Hybridization typically involves Southern analysis (Southern blotting), a method by which the presence of DNA sequences in a target nucleic acid mixture are identified by hybridization to a labeled oligonucleotide or DNA fragment probe. Southern analysis typically involves electrophoretic separation of DNA digests on agarose gels, denaturation of the DNA after electrophoretic separation, and transfer of the DNA to nitrocellulose, nylon, or another suitable membrane support for analysis with a radiolabeled, biotinylated, or enzyme-labeled probe as described in sections 9.37-9.52 of Sambrook et al., (1989) *Molecular Cloning*, second edition, Cold Spring Harbor Laboratory, Plainview; NY.

A polynucleotide can hybridize under moderate stringency conditions or, preferably, under high stringency

conditions to a KAS polynucleotide disclosed herein. High stringency conditions are used to identify nucleic acids that have a high degree of homology to the probe. High stringency conditions can include the use of low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC); 0.1% sodium lauryl sulfate (SDS) at 65°C. Alternatively, a denaturing agent such as formamide can be employed during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is the use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Moderate stringency conditions refers to hybridization conditions used to identify nucleic acids that have a lower degree of identity to the probe than do nucleic acids identified under high stringency conditions. Moderate stringency conditions can include the use of higher ionic strength and/or lower temperatures for washing of the hybridization membrane, compared to the ionic strength and temperatures used for high stringency hybridization. For example, a wash solution comprising 0.060 M NaCl/0.0060 M sodium citrate (4X SSC) and 0.1% sodium lauryl sulfate (SDS) can be used at 50°C, with a last wash in 1X SSC, at 65°C. Alternatively, a hybridization wash in 1X SSC at 37°C can be used.

Hybridization can also be done by Northern analysis (Northern blotting), a method used to identify RNAs that hybridize to a known probe such as an oligonucleotide, DNA

fragment, cDNA or fragment thereof, or RNA fragment. The probe is labeled with a radioisotope such as ^{32}P, by biotinylation or with an enzyme. The RNA to be analyzed can be usually electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe, using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook et al., *supra*.

A polynucleotide has at least about 70% sequence identity, preferably at least about 80% sequence identity, more preferably at least about 90% sequence identity to SEQ ID NO:1, 3, 5, 7, 9, 11, or 13. Sequence identity can be determined, for example, by computer programs designed to perform single and multiple sequence alignments.

A polynucleotide of the invention can be obtained by chemical synthesis, isolation and cloning from plant genomic DNA or other means known to the art, including the use of PCR technology carried out using oligonucleotides corresponding to portions of SEQ ID NO:1, 3, 5, 7-9, 11 or 13. Polymerase chain reaction (PCR) refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, incorporated herein by reference, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, and the like. Alternately, a cDNA library (in an expression

vector) can be screened with KAS-specific antibody prepared using peptide sequence(s) from hydrophilic regions of the KAS protein of SEQ ID NO:2 and technology known in the art.

A polypeptide of the invention comprises an isolated polypeptide having the deduced amino acid sequence of Figs. 2, 4, 6, 8, 10 and 12, as well as derivatives and analogs thereof. By "isolated" is meant a polypeptide that is expressed and produced in an environment other than the environment in which the polypeptide is naturally expressed and produced. For example, a plant polypeptide is isolated when expressed and produced in bacteria or fungi. Similarly, a plant polypeptide is isolated when its gene coding sequence is operably linked to a chimeric regulatory element and expressed in a tissue where the polypeptide is not naturally expressed. A polypeptide of the invention also comprises variants of the KAS polypeptides disclosed herein, as discussed above.

A full-length KAS coding sequence may comprise the sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. Alternatively, a chimeric full-length KAS coding sequence may be formed by linking, in-frame, nucleotides from the 5′ region of a first KAS gene to nucleotides from the 3′ region of a second KAS gene, thereby forming a chimeric KAS protein.

It should be appreciated that nucleic acid fragments having a nucleotide sequence other than the KAS sequences disclosed in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 will encode a polypeptide having the exemplified amino acid coding sequence of SEQ ID NO:2, 4, 6, 8, 10, 12 or 14, respectively. The degeneracy of the genetic code is well-known to the art; i.e., for many amino acids, there is more than one nucleotide triplet which serves as the codon for the amino acid.

It should also be appreciated that certain amino acid substitutions can be made in protein sequences without affecting the function of the protein. Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 3, pp. 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity.

A nucleic acid construct of the invention comprises a polynucleotide as disclosed herein linked to another, different polynucleotide. For example, a full-length KAS coding sequence may be operably fused in-frame to a nucleic acid fragment that encodes a leader sequence, secretory sequence or other additional amino acid sequences that amy be usefully linked to a polypeptide or peptide fragment.

A transgenic plant of the invention contains a nucleic acid construct as described herein. In some embodiments, a transgenic plant contains a nucleic acid construct that comprises a polynucleotide of the invention operably linked to at least one suitable regulatory sequence in sense orientation. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the expression level of the polynucleotide to which they are linked. Examples of regulatory sequences are known in the art and include, without limitation, minimal promoters and promoters of genes preferentially or

exclusively expressed in seeds or in epidermal cells of stems and leaves. Native regulatory sequences of the polynucleotides disclosed herein can be readily isolated by those skilled in the art and used in constructs of the invention. Other examples of suitable regulatory sequences include enhancers or enhancer-like elements, introns, 3' non-coding regions such as poly A sequences and other regulatory sequences discussed herein. Molecular biology techniques for preparing such chimeric genes are known in the art.

In other embodiments, a transgenic plant contains a nucleic acid construct comprising a partial or a full-length KAS coding sequence operably linked to at least one suitable regulatory sequence in antisense orientation. The chimeric gene can be introduced into a plant and transgenic progeny displaying expression of the antisense construct are identified.

One may use a polynucleotide disclosed herein for cosuppression as well as for antisense inhibition. Cosuppression of genes in plants may be achieved by expressing, in the sense orientation, the entire or partial coding sequence of a gene. See, e.g., WO 04\11516, incorporated herein by reference.

Transgenic techniques for use in the invention include, without limitation, *Agrobacterium*-mediated transformation, viral vector-mediated transformation electroporation and particle gun transformation. Illustrative examples of transformation techniques are described in U.S. Patent 5,204,253, (particle gun) and U.S. Patent 5,188,958 (*Agrobacterium*), incorporated herein by reference. Transformation methods utilizing the Ti and Ri plasmids of *Agrobacterium spp*. typically use binary-type vectors. Walkerpeach, C. et al., in Plant Molecular Biology

Manual, S. Gelvin and R. Schilperoort, eds., Kluwer Dordrecht, C1:1-19 (1994). If cell or tissue cultures are used as the recipient tissue for transformation, plants can be regenerated from transformed cultures by techniques known to those skilled in the art.

Techniques are known for the introduction of DNA into monocots as well as dicots, as are the techniques for culturing such plant tissues and regenerating those tissues. Monocots which have been successfully transformed and regenerated include wheat, corn, rye, rice, and asparagus. See, e.g., U.S. Patent Nos. 5,484,956 and 5,550,318, incorporated herein by reference.

For efficient production of transgenic plants from plant cells, it is desirable that the plant tissue used for transformation possess a high capacity for regeneration. Transgenic plants of woody species such as poplar and aspen have also been obtained. Technology is also available for the manipulation, transformation, and regeneration of gymnosperm plants. For example, U.S. Patent No. 5,122,466 describes the biolistic transformation of conifers, with preferred target tissue being meristematic and cotyledon and hypocotyl tissues. U.S. Patent No. 5,041,382 describes enrichment of conifer embryonal cells.

Seeds produced by a transgenic plant(s) can be grown and then selfed (or outcrossed and selfed) to obtain seeds homozygous for the construct. Seeds can be analyzed in order to identify those homozygotes having the desired expression of the construct. Transgenic plants may be entered into a breeding program, e.g., to introgress the novel construct into other lines, to transfer the construct to other species, or for further selection of other desirable traits. Alternatively, transgenic plants may be propagated vegetatively for those species amenable to such

techniques. A nucleic acid construct of the invention can alter the levels of very long chain fatty acids in plant tissues expressing the polynucleotide, compared to VLCFA levels in corresponding tissues from an otherwise identical plant not expressing the polynucleotide. A comparison can be made, for example, between a non-transgenic plant of a plant line and a transgenic plant of the same plant line. Levels of VLCFAs having 20-32 carbons and/or levels of VLCFAs having 32-60 carbons can be altered in plants disclosed herein. Plants having an altered VLCFA composition may be identified by techniques known to the skilled artisan, e.g., thin layer chromatography or gas-liquid chromatography (GLC) analysis of the appropriate plant tissue.

A suitable group of plants with which to practice the invention are the *Brassica* species, including *B. napus*, *B. rapa*, *B.juncea*, and *B. hirta*. Other suitable plants include, without limitation, soybean (*Glycine max*), sunflower (*Helianthus annuus*) and corn (*Zea mays*).

A method according to the invention comprises introducing a nucleic acid construct into a plant cell and producing a plant (as well as progeny of such a plant) from the transformed cell. Progeny includes descendants of a particular plant or plant line, e.g., seeds developed on an instant plant are descendants. Progeny of an instant plant include seeds formed on F_1, F_2, F_3, and subsequent generation plants, or seeds formed on BC_1, BC_2, BC_3, and subsequent generation plants.

Methods and compositions according to the invention are useful in that the resulting plants and plant lines have desirable alterations in very long chain fatty acid composition. Suitable tissues in which to express polynucleotides and/or polypeptides of the invention

include, without limitation, seeds, stems and leaves. Leaf tissues of interest include cells and tissues of the epidermis, e.g., cells that are involved in forming trichomes. Of particular interest are epidermal cells involved in forming the cuticular layer. The cuticular layer comprises various very long chain fatty acids and VLCFA derivatives such as alkanes, esters, alcohols and aldehydes. Altering the composition and amount of VLCFAs in epidermal cells and tissues may enhance defense mechanisms and drought tolerance of plants disclosed herein.

Polynucleotides of the invention can be used as markers in plant genetic mapping and plant breeding programs. Such markers may include RFLP, RAPD, or PCR markers, for example. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. Marker-assisted breeding techniques may be used in addition to, or as an alternative to, other sorts of identification techniques. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence from a desired KAS that has been introduced into a plant line and is being crossed into other plant lines.

Plants and plant lines disclosed herein preferably have superior agronomic properties. Superior agronomic characteristics include, for example, increased seed germination percentage, increased seedling vigor, increased resistance to seedling fungal diseases (damping off, root rot and the like), increased yield, and improved standability.

While the invention is susceptible to various modifications and alternative forms, certain specific embodiments thereof are described in the general methods and examples set forth below. It should be understood, however,

that these examples are not intended to limit the invention to the particular forms disclosed but, instead the invention is to cover all modifications, equivalents and alternatives falling within the scope of the invention.

EXAMPLES

Example 1

Cloning and Expression of FAE1 in Yeast Cells

The open reading frame of the *Arabidopsis FAE1* gene was amplified directly by PCR, using *Arabidopsis thaliana cv.* Columbia genomic DNA as a template, pfu DNA polymerase and the following primers: 5'CTCGAGGAGCAATGACGTCCGTTAA-3' and 5'-CTCGAGTTAGGACCGACCGTTTTG-3'. The PCR product was blunt-end cloned into the *Eco* RV site of pBluescript (Stratagene, La Jolla, CA),

The *FAE1* gene was excised from the Bluescript vector with Bam*H*1, and then subcloned into the pYEUra3 (Clontech, Palo Alto, CA). pYEUra3 is a yeast centromere-containing, episomal plasmid that is propagated stably through cell division. The *FAE1* gene was inserted downstream of a GAL1 promoter in pYEUra3. The GAL1 promoter is induced when galactose is present in the medium and repressed when glucose is present in the growth medium.

Insertion of the *FAE1* gene in the sense orientation was confirmed by PCR, and pYEUra3/*FAE1* was used to transform *Saccharomyces cerevisiae* strain AB1380 using a lithium acetate procedure as described in Gietz, R. and Woods, R., in Molecular Genetics of Yeast: Practical Approaches, Oxford Press, pp. 121-134 (1994). Plasmid DNA was isolated from putative transformants, and the presence of the *FAE1*/pYEUra3 construct was confirmed by Southern analysis.

Yeast transformed with pYEUra3 having *FAE1* operably linked to the GAL1 promoter were grown in the presence of

galactose or glucose and were analyzed for the expression of *FAE1*. As a control, yeast transformed with pYEUra3 containing no insert were also assayed. Analysis of such control preparations yielded fatty acid compositions and fatty acid elongation rates similar to those of yeast transformed with pYEUra3/*FAE1* and grown with glucose as the carbon source.

The fatty acid composition of yeast cells grown in the presence of galactose was compared to that of cells grown in the presence of glucose, to determine if VLCFA were found in the galactose-induced cells.

Transformed yeast cells were grown overnight in YPD media at 30°C with vigorous shaking. One hundred μl of the overnight culture were used to inoculate 40 ml of complete minimal uracil dropout media (CM-Ura) supplemented with either glucose or galactose (2% w/v). Cultures were grown at 30°C to an OD_{600} of approximately 1.3 to 1.5. Cells were harvested by centrifugation at 5000 Xg for 10 min. Total lipids were extracted from the cells with 2 volumes of 4N KOH in 100% methanol for 60 min. at 80°C. Fatty acids were saponified and methyl esters were prepared by drying the samples and resuspending in 0.5 ml of boron trichloride in methanol (10% v/v). Samples were incubated at 50°C for 15 min in a sealed tube. About 2 ml of water was then added and the fatty methyl esters were extracted thrice with 1 ml of hexane. Extracts were dried under nitrogen and redissolved in hexane. See Hlousek-Radojcic, A. et al., Plant J. 8:803-809. Methyl esters were analyzed on an HP 5890 series II gas chromatograph equipped with a 5771MSD and 7673 auto injector (Hewlett-Packard, Cincinnati, OH). Methyl esters were separated on a DB-23 (J&W Scientific) capillary column (30 m X 0.25 mm X 0.25 μm). The column was operated with helium carrier gas and splitless injection

(injection temperature 280°C, detector temperature 280°C). After an initial 3 min. at 100°C, the oven temperature was raised to 250° at 20°C min^{-1} and maintained at that temperature for an additional 3 min. The identity of the peaks was verified by cochromatography with authentic standards and by mass spectrometer analysis.

The results clearly revealed the appearance of both 20:1 and 22:1 acyl-CoA products in galactose-induced yeast containing the *FAE1* coding sequence. Uninduced yeast cells failed to accumulated significant amounts of fatty acids longer than C18. These results indicate that expression of *FAE1* in yeast resulted in functional KAS activity and functional elongase activity.

Example 2

FAE1 Activity in Yeast Microsomes

The functional expression of the *FAE1* KAS was analyzed by isolating microsomes from transformed yeast cells and assaying these microsomes *in vitro* for elongase activity.

Transformed yeast cells were grown in the presence of either glucose or galactose (2% w/v) as described in Example 1. Cells were harvested by centrifugation at 5000 Xg for 10 min and washed with 10 ml ice cold isolation buffer (IB), which contains 80 mM Hepes-KOH, pH 7.2, 5 mM EGTA, 5 mM EDTA, 10 mM KCl, 320 mM sucrose and 2 mM DTT). Cells were then resuspended in enough IB to fill a 1.7 ml tube containing 700 µl of 0.5 µm glass beads and yeast microsomes were isolated from the cells essentially as described in Tillman, T. and Bell, R., J. Biol. Chem. 261:9144-9149 (1986). The microsomal membrane pellet was recovered by centrifugation at 252,000 xg for 60 min. The pellet was rinsed by resuspending in 40 ml fresh IB and

again recovered by centrifugation at 252000 Xg for 60 min. Microsomal pellets were resuspended in a minimal volume of IB, and the protein concentration adjusted to 2.5 $\mu g\ \mu l^{-1}$ by addition of IB containing 15% glycerol. Microsomes were frozen on dry ice and stored at -80°C. The protein concentration in microsomes was determined by the Bradford method (Bradford, 1976).

Fatty acid elongase activity was measured essentially as described in Hlousek-Radojcic, A. et al., Plant J. 8:803-809 (1995). Briefly, the standard elongation reaction mix contained 80 mM Hepes-KOH, pH 7.2, 20 mM $MgCl_2$, 500 μM NADPH, 1 mM ATP, 100 uM malonyl-CoA, 10 μM CoA-SH and 15 μM radioactive acyl-CoA substrate. The radiolabeled substrate was either [1-^{14}C]18:1-CoA (50 uCi μmol^{-1}), [1-^{14}C]18:0-CoA (55 uCi μmol^{-1}), or [1-^{14}C]16:0-CoA (54 uCi μmol^{-1}). The reaction was initiated by the addition of yeast microsomes (5 μg protein) and the mixture incubated at 30° C for the indicated period of time. The final reaction volume was 25 μl.

Methyl esters of the acyl-CoA elongation products were prepared as described in Example 1. Methyl esters were separated on reversed phase silica gel KC18 TLC plates (Whatman, 250 uM thick), quantified by phosphorimaging, and analyzed on by ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA). The detection limit for each product is about 0.001 nanomoles per min. per mg microsomal protein, depending on the phosphorimage exposure time.

Results of representative *in vitro* elongation assays are shown in Figs. 1 and 2. The results indicate that microsomes from galactose-induced cells expressing *FAE1* catalyzed multiple cycles of elongation starting with either C16:0 acyl CoA, C18:0 acyl CoA, or C18:1 acyl-CoA as the substrate (Fig. 1). The 16:0 and 18:0 acyl-CoA substrates

were elongated to C26:0 acyl-CoA. In contrast, the 18:1-CoA substrate was elongated primarily to C20:1, with only low levels of C22:1 acyl-CoA being produced. Occasionally, trace levels of C24:1 CoA were also observed. Although the chain length of the products from the 18:1 acyl-CoA substrate were less than the chain length from the saturated acyl-CoA substrates, the rate of elongation of oleoyl-CoA was about 2- and 3-fold higher than the rates of elongation of 16:0-CoA and 18:0-CoA, respectively.

The elongation activity observed in microsomes from uninduced cells indicated that there was a low level of endogenous elongase activity when 18:1-CoA or 18:0-CoA were used as substrates. There was substantial 16:0-CoA elongase activity (10.1 nmol mg protein^{-1} at 30 min) in microsomes from uninduced cells (Fig. 2). However, the major product of 16:0 elongation using uninduced microsomes was C18:0 acyl CoA, with only small amounts of products beyond this length. The elongation of the 16:0 acyl-CoA substrate presumably is due to an endogenous yeast elongase.

Elongation of 18:1 CoA by microsomes from induced cells occurred at a rate about 18-fold higher than in microsomes isolated from the uninduced cells (Fig. 2). With microsomes from induced yeast, synthesis of 20:0 CoA from the 16:0 CoA substrate, occurred at a rate similar to that seen when the substrate was 18:0 CoA (4.2 vs. 5.1 nmol mg protein^{-1}). The total rate of elongation of [^{14}C] 16:0-CoA by microsomes from induced cells (15.8 nmol mg protein^{-1} at 30 min.) was more than 50% higher than elongation of [^{14}C] 16:0-CoA by microsomes from uninduced cells, suggesting that the *FAE1* KAS utilized 16:0-CoA as a substrate in addition to C18-C24 acyl-CoAs. The *FAE1* elongase KAS activity, i.e., the difference in the 16:0 elongation rates between microsomes from induced and uninduced cells, was 5.7 nmol mg

protein^{-1}. The elongation rate with the 16:0 substrate thus was similar to the elongase activity of the *FAE1* elongase KAS with the 18:0 substrate.

These results indicate that *FAE1* KAS expressed in yeast could synthesize 3-ketoacyl-CoA *in vitro* and, in combination with yeast reductases and dehydrases, could form a functional VLCFA elongase complex. In addition, these results suggest that *FAE1* is membrane-bound in yeast cells.

Example 3

Cloning and Sequencing of *Arabidopsis* Elongase Genes

The sequence of a jojoba seed cDNA (see WO 93/10241 and WO 95/15387, incorporated herein by reference) was used to search the Arabidopsis expressed sequence tag (EST) database of the Arabidopsis Genome Stock Center (The Ohio State University, Columbus, Ohio). The BLAST computer program (National Institutes of Health, Bethesda, MD, USA) was used to perform the search. The search identified two ESTs (ATTS1282 and ATTS3218) that had a high degree of sequence identity with the jojoba sequence. The ATTS1282 and ATTS3218 ESTs appeared to be partial cDNA clones rather than full-length clones based on the length of the jojoba sequence.

A genomic DNA library from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda GEM11 vector (Promega, Madison, Wisconsin) and was obtained from Ron Davis, Stanford University, Stanford, CA. The library was hybridized with ATTS1282 and ATTS3218 as probes and 2 clones were identified for each EST. Phage DNA was isolated from each of the hybridizing clones, the genomic insert was excised with the restriction enzyme Sac I and subcloned into the plasmid pBluescript (Stratagene, La Jolla, CA). One clone from the ATTS1282 hybridization was designated EL1 and

one clone from the ATTS3218 hybridization was designated EL2.

A yeast expression library, containing cDNA from *Arabidopsis thaliana* cv. Columbia, was prepared in the lambda YES expression vector described in Elledge et al. (Elledge, S. et al., Proc. Natl. Acad. Sci USA 88:1731-1735 (1991) and was obtained from Ron Davis at Stanford University, Stanford, CA. The library was hybridized with a EL2 partial cDNA probe. A full-length EL2 cDNA was not identified. However, the probe did identify a full-length cDNA which was designated EL3.

A consensus sequence for the C-terminal region of EL1, EL2 and the jojoba cDNA polypeptides was identified by sequence alignment using DNA analysis programs from DNAStar, Madison, Wisconsin. This consensus sequence was used to search the *Arabidopsis* EST database again for ß-keto acyl synthase sequences. These searches identified four additional putative ß-keto acyl synthase ESTs, which were designated EL4 through EL7. EL4, EL5, EL6, and EL7 have homology to Genbank Accession Nos. T04345, T44939, T22193 and T76700, respectively.

The lambda YES cDNA expression library described above was hybridized with the EL1 and EL4-EL7 ESTs as probes. This screen identified full-length cDNAs for EL1, EL5 and EL6.

The lambda GEM11 genomic library was hybridized with the EL4 and EL7 ESTs as probes. This screen identified full-length genomic clones for EL4 and EL7. Phage DNA was isolated from each of the hybridizing clones and subcloned into pBluescript as described above.

The 7 EL clones were sequenced on both strands with regions of overlap for each sequence run. Sequencing was carried out with an ABI automated sequencer (Applied

Biosystems, Inc., Foster City, California), following the manufacturer's instructions.

The nucleotide sequences for the coding regions of EL1-EL7 are shown in Figs. 3, 5, 7, 9, 11, 13 and 15, respectively. The deduced amino acid sequences for EL1-EL7 are shown in Figs. 4, 6, 8, 10, 12, 14 and 16, respectively, using the standard one-letter amino acid code. The EL1, EL2 and EL7 genomic clones appeared to lack introns. The EL4 genomic clone contained one intron near the 5' end of the coding region.

The nucleotide sequences of the 7 EL polynucleotides were compared to 5 DNA sequences present in Genbank. Genbank, National Center for Biotechnology Information, Bethesda, MD. Two of the 5 accessions were cloned from members of the Brassicaceae: the *Arabidopsis* FAE1 sequence (Accession U29142) and a *Brassica napus* sequence (Accession U50771). Three of the accessions were cloned from jojoba (*Simmondsia chinensis*): 2 wax biosynthesis genes (Accessions I14084 and I14085) and a jojoba KAS gene (Accession U37088). See also U.S. Patent 5,445,947, incorporated herein by reference.

Multiple alignment of the 12 sequences was carried out with a computer program sold under the trade name MEGALIGN Lasergene by DNAStar (Madison, Wisconsin). Alignments were done using the Clustal method with weighted residue weight table. The nucleotide sequence similarity index and percent divergence based on the multiple alignment algorithm is shown in Table 1. The nucleotide sequences of EL1-EL7 are distinguishable from the 5 DNA sequences obtained from Genbank.

The deduced amino acid sequences of the EL1-7 polypeptides were compared with the MEGALIGN program to the deduced amino acid sequences of the same 5 Genbank clones,

using the Clustal method with PAM250 residue weight table. The amino acid sequence similarity and percent divergence are shown in Table 2. The amino acid sequences of EL1-EL7 polypeptides are distinguishable from those of the Genbank sequences.

TABLE 1

Nucleotide sequence pair distances of EL1-EL7, using Clustal method with weighted residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12		
1		77.5	62.4	58.8	57.0	54.9	47.0	42.8	42.9	43.1	44.7	41.3	1	ARAFAE1 U29142
2	18.1		61.0	57.9	55.4	53.7	46.9	42.7	44.1	42.9	42.3	40.5	2	BNaFAE1 U50771
3	40.4	41.0		70.5	59.3	56.4	46.7	48.5	48.1	48.6	46.5	43.5	3	EL2
4	43.9	44.3	28.0		56.3	55.4	46.5	47.0	45.1	47.2	47.4	42.3	4	EL3
5	40.7	42.3	45.0	45.0		68.0	54.0	46.8	46.6	46.4	49.0	47.2	5	EL5
6	45.8	48.9	46.0	47.3	32.4		53.6	48.6	48.2	48.6	49.0	49.2	6	EL7
7	74.1	71.0	69.4	67.3	64.3	64.5		49.8	49.2	49.8	47.7	48.2	7	EL6
8	68.1	66.2	63.4	63.1	65.5	64.2	56.1		97.7	99.7	48.4	45.8	8	JOJOKCS U37088
9	67.0	65.4	63.7	64.6	64.6	64.1	56.6	1.1		95.9	47.6	44.8	9	JOKCS10 I14084
10	67.2	65.2	61.8	61.4	64.1	63.0	56.3	0.2	1.1		48.4	45.3	10	JOKCS11 I14085
11	88.6	85.8	81.0	77.0	77.4	82.4	83.1	71.1	71.1	69.9		48.3	11	EL1
12	95.7	90.4	95.4	91.5	84.5	82.8	91.9	73.4	73.8	73.3	59.9		12	EL4
	1	2	3	4	5	6	7	8	9	10	11	12		

TABLE 2

Amino acid sequence pair distances of EL1-EL7, using Clustal method with PAM250 residue weight table.

	1	2	3	4	5	6	7	8	9	10	11	12		
1		72.0	62.9	59.8	60.9	60.2	50.3	51.9	52.1	51.5	49.1	42.0	1	EL2
2	31.1		60.1	57.5	58.7	57.1	49.8	49.8	50.0	49.2	49.6	44.4	2	EL3
3	47.4	48.7		82.4	60.7	63.0	50.0	51.4	51.6	50.8	47.8	43.9	3	ATFAE1 U29142
4	51.8	52.8	17.9		60.2	61.0	49.2	50.3	50.5	49.7	46.5	42.4	4	BNFAE1 U50771
5	49.0	51.3	45.8	46.2		75.8	61.0	58.7	58.9	58.3	55.0	55.6	5	EL7
6	52.6	55.5	42.8	46.5	29.3		61.8	55.7	55.7	54.9	52.9	50.5	6	EL5
7	74.7	70.5	71.8	74.4	52.0	50.8		52.8	52.8	51.8	53.4	51.6	7	EL6
8	66.7	69.2	66.2	67.3	54.8	59.8	67.7		99.8	96.9	53.1	52.0	8	JOJKCS U37088
9	66.3	68.7	66.2	67.3	54.0	59.3	67.7	0.2		96.9	53.1	51.9	9	JKCS11 I14085
10	66.3	69.7	66.6	67.8	54.5	60.7	68.6	1.8	1.6		51.7	50.7	10	JKCS10 I14084
11	73.6	73.7	72.8	74.4	60.8	66.0	67.2	63.9	63.9	65.3		50.8	11	EL1
12	84.8	85.5	82.7	83.3	60.6	70.8	67.1	68.5	68.5	69.9	69.4		12	EL4
	1	2	3	4	5	6	7	8	9	10	11	12		

Example 4

Expression of EL1 and EL2 in Yeast

The open reading frames (ORFs) for the EL2, EL4 and EL7 clones were amplified by PCR. The EL2 ORF was cloned into λYES using the primers: CTCGAGCAAGTCCACTACCACGCA and CTCGAGCGAGTCAGAAGGAACAAA. The EL4 ORF was cloned into pYEUra3 using the primers: GATAATTTAGAGAGGCACAGGGT and GTCGACACAAGAATGGGTAGATCCAA. The EL7 ORF was cloned into pYEUra3 using the primers: CAGTTCCTCAAACGAAGCTA and GTCGACTTCTCAATGGACGGTGCCGGA. Amplified products were cloned into pYEUra3 under the control of, and 3′ to, the GAL1 promoter. The resulting plasmids were transformed into yeast as described in Example 1.

Yeast cultures containing full-length EL1 in λYES and full-length EL2 in pYEUra3 were grown in the presence of galactose or glucose as described in Example 2. Microsomes were then prepared from each of the cultures and fatty acid elongation assays were carried out as described in Example 2.

In the first experiment, microsomes were prepared from galactose-induced cultures of EL1, EL2 and FAE1, and incubated with either [1-^{14}C] 18:0 acyl-CoA or [1-^{14}C] 18:1 acyl-CoA as substrate. The amounts of various reaction products synthesized after 30 minutes (min) were determined as described in Example 2. The results when 18:0 acyl-CoA was the substrate are shown in Table 3. The results when 18:1 acyl-CoA was the substrate are shown in Table 4.

Table 3.
Elongation of 18:0-CoA by FAE1, EL1 and EL2 Genes Expressed in Yeast

	ß-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
Acyl-CoA Product	Rate[1]	(%)	Rate	(%)	Rate	(%)
20:0	0.369	64.3	0.084	38.8	0.108	41.8
22:0	0.113	18.6	0.047	21.9	0.053	20.7
24:0	0.065	10.7	0.043	19.9	0.052	20.3
26:0	0.038	6.3	0.042	19.4	0.044	17.2
Total	0.585	100.0	0.216	100.0	0.258	100.0

[1] Nanomoles/minute/mg of microsomal protein

Table 4.
Elongation of 18:1-CoA by FAE1, EL1 and EL2 Genes Expressed in Yeast

	ß-Keto Acyl Synthase Gene					
	FAE1		EL1		EL2	
Acyl-CoA Product	Rate[1]	(%)	Rate	(%)	Rate	(%)
20:1	1.131	84.6	0.111	80.8	0.091	84.1
22:1	0.206	15.4	0.026	19.2	0.017	15.9
24:1	0.0	0.0	0.0	0.0	0.0	0.0
26:1	0.0	0.0	0.0	0.0	0.0	0.0
Total	1.337	100.0	0.137	100.0	0.108	100.0

[1] Nanomoles/minute/mg of microsomal protein

The results shown in Tables 3 and 4 indicate that the EL1 and EL2 gene products have ß-ketoacyl synthase (KAS) activity and that the KAS reaction product can be utilized to form VLCFAs. The specific activities of the 3 KAS enzymes cannot be compared, since the relative amount of the heterologous KAS protein in each microsomal preparation is not known. However, the proportions of various reaction products can be compared between FAE1, EL1 and EL2.

The data shown in Table 3 indicate that the EL1 and EL2 KAS activities result in a higher proportion of saturated VLCFAs than does the FAE1 KAS activity. These results suggest that EL1 and EL2 encode novel gene products, because EL1 and EL2 have a greater preference for C22:0 and C24:0 acyl-CoA substrates than does *FAE1*.

A comparison of the relative elongation activity of FAE1 with 18:0 and 18:1 substrates (Tables 3 and 4) indicates that FAE1 is more active when 18:1 is the

substrate than when 18:0 is the substrate. In contrast, the overall rate of product formation with EL1 is less when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). EL2 is also less active when 18:1 is the substrate than when 18:0 is the substrate (Tables 3 and 4). These results support the conclusion that EL1 and EL2 encode novel gene products and suggest that EL1 and EL2 have a preference for saturated fatty acids as substrates, whereas the FAE1 gene product has a preference for monounsaturated fatty acids as substrates.

In a second experiment, microsomes were prepared from galactose-induced and from glucose-repressed yeast cultures containing EL1 or EL2 coding sequences. The microsomal preparations were incubated with either 18:0 acyl-CoA or 18:1 acyl-CoA as substrate and the fatty acid reaction products determined as described above. The results with the 18:0 substrate are shown in Table 5. The results with the 18:1 substrate are shown in Table 6.

Table 5.
Elongation of 18:0-CoA by EL1 and EL2
With and Without Induction of Gene Expression

	ß-Keto Acyl Synthase Gene							
	EL1				EL2			
Acyl	+Glucose		+Galactose		+Glucose		+Galactose	
CoA	Rate[1]	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:0	0.007	100.0	0.074	55.8	0.030	81.3	0.107	43.1
22:0	0.000	0.0	0.023	17.4	0.002	5.1	0.044	17.8
24:0	0.000	0.0	0.020	15.3	0.005	13.6	0.048	19.1
26:0	0.000	0.0	0.015	11.5	0.000	0.0	0.050	20.0
Total	0.007	100.0	0.133	100.0	0.037	100.0	0.249	100.0

[1] Nanomoles/minute/mg of microsomal protein

Table 6.
Elongation of 18:1-CoA by EL1 and EL2
With and Without Induction of Gene Expression

	ß-Keto Acyl Synthase Gene							
	EL1				EL2			
Acyl CoA	+Glucose		+Galactose		+Glucose		+Galactose	
	Rate[1]	(%)	Rate	(%)	Rate	(%)	Rate	(%)
20:1	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0
22:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
24:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
26:1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
Total	0.062	100.0	0.081	100.0	0.043	100.0	0.089	100.0

[1] Nanomoles/minute/mg of microsomal protein

The results in Table 5 show *in vitro* elongase activity for EL1 and EL2 under induced (galactose) and uninduced (glucose) conditions. The comparison indicates that induction with galactose results in a large increase in overall elongase activity when 18:0 acyl CoA is the substrate (about 19-fold and 7-fold for EL1 and EL2, respectively). In contrast, induction when 18:1 acyl CoA is the substrate results in only a small increase in elongase activity (about 1.3-fold and 2-fold for EL1 and El2, respectively), as shown in Table 6.

The results in Table 5 show that little or no VLCFA products are made by yeast microsomes under uninduced conditions. Upon induction of EL1 and EL2 gene expression, however, significant quantities of C20:0, C22:0, C24:0 and C26:0 are made. The data in Tables 5 and 6 are consistent with the results in Tables 3 and 4, which indicated that EL1 and EL2 were more active with a saturated fatty acid substrate than with a monounsaturated substrate.

The data in Tables 5 and 6 are also consistent with the data in Tables 3 and 4 indicating that the EL1 and EL2 gene products are more active in converting C24:0 to C26:0 than is *FAE1*.

In a third experiment, microsomes from induced and uninduced cultures containing EL1 or EL2 were incubated in the absence of cofactors involved in the ß-ketoacyl condensation reaction. Cultures were induced and microsomes were prepared as described in Example 2. *In vitro* assays were carried out as described in Example 2, except that either ATP, CoASH or both were omitted from the enzyme reaction mixture. In addition, one reaction was carried out in a complete mixture having 0.01 mM of cerulenin (Sigma, St. Louis, MO). Cerulenin is an inhibitor of some condensing enzymes. The results are shown in Tables 7-9.

Table 7.
Effect of Cofactors on 18:0-CoA Elongation[1]

Gene	Expt[4]	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+ Cer[3]
EL1	1	.037	.109	.095	.105	.119	.141
	2	N.D.	.090	.125	.093	.270	.176
EL2	1	.033	.112	.168	.127	.143	.238
	2	N.D.	.120	.178	.133	.195	.302

[1] Activity in nanomoles/minute/mg of microsomal protein.
[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.
[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.
[4] Experiment No.

Table 8.

Effect of Cofactors on Elongation Products of EL1[1]

Prod.	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+Cer[3]
20:0	53.9	46.2	34.4	47.8	41.7	46.7
22:0	14.4	18.7	13.7	18.0	19.4	16.2
24:0	18.5	18.1	20.6	19.1	16.7	17.7
26:0	13.2	17.1	31.4	15.2	22.3	19.4
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.
[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.
[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

Table 9.

Effect of Cofactors on Elongation Products of EL2[1]

Prod.	+Glu[2]	+Gal[2]	-ATP[3]	-CoA[3]	-A&C[3]	+Cer[3]
20:0	54.5	47.1	34.1	45.3	38.0	41.8
22:0	17.1	19.1	16.4	19.2	15.9	16.1
24:0	5.8	19.4	20.8	19.9	18.4	20.4
26:0	22.6	14.5	28.9	15.8	27.8	21.8
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1] Amount of indicated product as a percent of total products formed. Results of one experiment for +Glucose; Average of two experiments for other conditions.
[2] +Glu: microsomes from cultures grown in the presence of glucose and incubated in standard reaction mix; +Gal: microsomes from cultures grown in the presence of galactose and incubated in standard reaction mix.
[3] Microsomes from galactose-induced cultures. -ATP: ATP omitted from reaction mix; -CoA: Coenzyme A omitted from reaction mix; -A&C: ATP and Coenzyme A omitted from reaction mix; +Cer: Standard reaction mix containing 0.01 mM cerulenin.

The results in Table 7 indicate that omission of ATP and/or CoA from the incubation mixture does not have a significant effect on the overall amounts of VLCFAs synthesized by the *in vitro* KAS activity of EL1 or EL2. The results also show that cerulenin does not inhibit the KAS activity of EL1 or EL2. The data in Table 8 and 9 confirm that EL1 and EL2 KAS activity produces significant amounts of C24:0 and C26:0 acyl CoA products.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those skilled in the art without deviating from the spirit and scope of the appended claims.